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# MERCURY COMPOUNDS IN THE CHEMOTHERAPY OF EXPERIMENTAL TUBERCULOSIS IN GUINEA-PIGS. I

STUDIES ON THE BIOCHEMISTRY AND CHEMOTHERAPY  
OF TUBERCULOSIS. XXI

LYDIA M. DEWITT

*From the Otho S. A. Sprague Memorial Institute and the Pathological Laboratory of the  
University of Chicago*

Perhaps no drug has a longer history of use in therapy than has mercury. Much of this history, and especially that of the newer organic mercury compounds, has been reviewed by Schamberg, Kolmer and Raiziss<sup>1</sup> and by others, and need not receive further attention here. In tuberculosis, mercury in various forms and by various methods has been used to a greater or less extent clinically since the time of Paracelsus early in the sixteenth century. There have been certain periods when it was in especial favor and others when it was in disrepute. Hall,<sup>2</sup> while not regarding it as a specific, thinks that small doses of mercurial preparations are "our most potent chemical weapons against this disease." Many others at about the same period, published favorable reports on mercury therapy of tuberculosis. In 1908 and 1909 Barton L. Wright<sup>3</sup> reported a considerable number of cases treated with mercuric succinimide and potassium iodide alternately, and his favorable reports were followed by many others, who used the same or similar treatment. The general consensus of opinion seems to be that mercurials have a good effect on the condition of the patient, but that mercury is in no sense a specific in tuberculosis.

Only one report in the literature has been found by me on the use of mercury in experimental tuberculosis in animals. George Cornet<sup>4</sup> tested mercuric chloride among a series of disinfectants, in 8 guinea-pigs. He injected mercuric chloride for 10 days until the animals began to show toxic effects; he then inoculated them with tubercle bacilli. He was unable to note any difference in the extent

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<sup>1</sup> Am. J. Syphilis, 1917, 1, p. 1.

<sup>2</sup> Am. J. Med. Sc., 1889, 98, p. 45.

<sup>3</sup> New York Med. Jour., 1909, 89, p. 1180.

<sup>4</sup> Ztschr. f. Hyg. u. Infektionskr., 1889, 98, p. 98.

of the disease between the mercurialized animals and the controls. Some died of mercurial poisoning early, and all the others showed generalized tuberculosis.

Robert Koch<sup>5</sup> stated that mercury in vapor form inhibited the growth of the tubercle bacillus in the test tube but was entirely without influence on the progress of the disease in animals.

In view of the long continued and repeatedly favorable use of mercurials in the clinical treatment of human tuberculosis and the paucity of experimental evidence as to their value, it seemed advisable, in connection with the systematic investigations on the chemotherapy of tuberculosis carried on in this laboratory, that the value of mercury and its compounds should be investigated in considerable detail.

In the internal use of compounds of mercury, it is necessary to consider (1) the stability of the preparation in the animal tissues, (2) its method and form of absorption and excretion, (3) the place of deposition of the part of the compounds which is not excreted, and (4) the toxicity and pathogenicity of the preparations.

Morley<sup>6</sup> states (1) that French workers claim that mercurials are converted in the stomach and intestines into mercuric chloride and circulate as the double salt of mercury and sodium; (2) that it is proved that mercury is absorbed and escapes as an albuminate in every excretion of the body, especially in the urine; (3) that a single dose is excreted within 24 hours; (4) that it accumulates, if given in small doses, and is deposited in all the organs. Blumenthal and Oppenheim<sup>7</sup> state that after the organic compounds of mercury used by them, a deposit of mercury was found constantly in the liver; sometimes in the intestines and occasionally in the lungs and in the blood. Schamberg, Kolmer and Raiziss<sup>8</sup> state (1) that the toxicity of mercurial salts is directly proportionate to the amount of mercury contained, the differences of molecular structure being of relatively little importance as affecting their toxicity; (2) that insoluble preparations injected intramuscularly are absorbed at the rate of a little over 1% per day; (3) that after 6 or 7 weeks, almost 50% of the mercury of insoluble preparations may be unabsorbed at the site of injection; and (4) that mercury has a great affinity for the cells of the kidney, and that this organ is one of the earliest involved in mercurial intoxication. All other workers do not agree with Schamberg, Kolmer and Raiziss on the relation of molecular structure to toxicity.

The bactericidal action of mercury has long been recognized, but its use as an internal antiseptic has been limited by its toxicity. Hence the efforts of chemists have for years been turned largely to the construction of mercuric compounds which should be nontoxic or less toxic than mercuric chloride, which kills, on the average, in intravenous doses above 4 mg. per kilogram of body weight, according to Sansum.<sup>9</sup> Abelin<sup>10</sup> states that the toxic influ-

<sup>5</sup> Vehr. d. X Internat. Med. Kong., 1890-91, I, Berlin. klin. Wchnschr., 1890, 27, p. 736.

<sup>6</sup> Calif. State Med. Jour., 1909, 7, p. 338.

<sup>7</sup> Biochem. Ztschr., 1914, 65, p. 460.

<sup>8</sup> Boston Med. & Surg. Jour., 1915, 162, p. 826.

<sup>9</sup> Jour. Am. Med. Assn., 1918, 70, p. 824.

<sup>10</sup> Deutsch. med. Wchnschr., 1912, 38, p. 1822.

ence of mercury compounds is in certain relation with their chemical structure. that the toxicity can be diminished by introduction of sulpho or sulph-amino groups or through double carbon connection of mercury, and that easily ionizable mercury compounds are more toxic than the less easily ionizable. Schrauth and Schoeller<sup>11</sup> showed that the disinfectant powers of organic mercury compounds were increased by substitution of the less acid phenolic hydroxyl for the carboxyl. They also showed that of the three isomeric mercuriated cresols, the meta derivative is the most potent disinfectant, while the ortho-hydroxy-mercuriphenoxide is more active than the para compound. Also, the entrance of a second hydroxymercuri group increases disinfectant power.

In the work, then, with new mercury compounds it has been necessary to keep the following factors in mind:

1. Minimal toxicity for animal tissues, i. e., organotropism.
2. Maximal disinfectant power
3. Stability or minimal ionization
4. Solubility in nontoxic and nonirritant solvents

This report, which will be followed by others on the same subject, concerns the bacteriostatic and therapeutic effect of some twenty-four inorganic and organic compounds of mercury on the human tubercle bacillus and on experimental tuberculosis in guinea-pigs.

Table 1 gives a list of the compounds reported in this paper, with their chemical formulae, when this was available, and their computed percentage content of mercury.

Part of these compounds have been purchased from Merck and Company, while many have been made for me by the following assistants working in the chemical laboratory of the University of Chicago: Walter Frankel, C. E. Cutler, S. M. Cadwell, Gladys Leavell, L. M. Larsen, Morris Kharasch, Friedrich Lommen, I. M. Jacobsohn.

The inhibitory or bacteriostatic power of most of these mercurials was tested in the usual way by adding the required amount of stock solution of the compound to tubes of melted agar and shaking until well mixed; the tubes were then slanted and cooled and inoculated with human tubercle bacilli, control tubes being made from the same culture. Certain of the compounds could not be dissolved in hot or cold water or in hot agar. Some of these could be dissolved in dilute alkali, in which case the stock solutions were made in either normal sodium hydroxide or 5% sodium carbonate (anhydrous) and diluted with water twenty times. The same amount of the alkali was also added to the control tubes to determine whether the alkalinity used had any inhibitory effect on the growth of the organisms. Some of the compounds, as shown in column 2 of table 2, were insoluble in any known solvent that could be used in this experiment. The required

<sup>11</sup> Ztschr. f. Hyg. u. Infektionskr., 1916, 82, p. 279.

TABLE 1

Name of Compound	Percentage of Mercury	Chemical Formula
Mercurous Chloride .....	84.92	$(\text{Hg}-\text{Cl})_2$
Mercuric Chloride .....	93.78	$\text{Hg}-\begin{array}{c} \text{Cl} \\   \\ \text{Cl} \end{array}$
Mercuric Sulpho-Cyanide .....	63.29	$\text{Hg}-\begin{array}{c} \text{S}-\text{C}\equiv\text{N} \\   \\ \text{S}-\text{C}\equiv\text{N} \end{array}$
Mercury Potassium Cyanide.....	68.72	$\text{Hg}(\text{CN})_2 \cdot 2 \text{KCN}$
Allyl Alcohol Mercuric Acetate.....	63.3	$\begin{array}{c} \text{H}-\text{C}-\text{Hg}-\text{O}-\text{C}-\text{CH}_3 \\   \quad \quad \quad   \\ \text{H}-\text{C} \quad \quad \quad \text{O} \\   \\ \text{H}-\text{C}-\text{OH} \end{array}$
Mercuric Succinimide .....	50.5	$\begin{array}{c} \text{H}-\text{C}-\text{C}=\text{O} \\   \quad \quad \quad   \\ \text{H}-\text{C} \quad \quad \quad \text{N} \\   \quad \quad \quad   \\ \text{H}-\text{C}-\text{C}=\text{O} \end{array} \cdot \text{Hg}$
Mercuric Salicylate .....	42.2	$\begin{array}{c} \text{HO}-\text{C}_6\text{H}_4-\text{C}(=\text{O})-\text{O}-\text{Hg}-\text{O}-\text{C}(=\text{O})-\text{C}_6\text{H}_4-\text{OH} \end{array}$
Mercurio-Iodo Hemol (Merck).....	12.0	Patented Preparation of Hemoglobin, Mercury and Iodine
Mercuriol (Merck) .....	10.0	Patented Preparation of Mercury and Nucleic Acid
Mercury Phenolphthalein .....	39.0	$\begin{array}{c} \text{C}_6\text{H}_5-\text{C}(\text{C}_6\text{H}_4-\text{O})_2-\text{C}(=\text{O})-\text{O}-\text{Hg}-\text{O}-\text{C}(=\text{O})-\text{C}_6\text{H}_5 \end{array}$
Mercury Tetraiodophenolphthalein ...	19.6	$\begin{array}{c} \text{C}_6\text{H}_5-\text{C}(\text{C}_6\text{H}_4-\text{O})_2-\text{C}(=\text{O})-\text{O}-\text{Hg}-\text{O}-\text{C}(=\text{O})-\text{C}_6\text{H}_5 \end{array}$
Fluorescein Mercuric Chloride.....	33.17	$\begin{array}{c} \text{C}_6\text{H}_5-\text{C}(\text{C}_6\text{H}_4-\text{O})_2-\text{C}(=\text{O})-\text{O}-\text{Hg}-\text{O}-\text{C}(=\text{O})-\text{C}_6\text{H}_5 \end{array} \cdot \text{HgCl}_2$
Trypan Blue Mercuric Chloride.....	15.65	$\begin{array}{c} \text{N}_4\text{O}_5\text{S} \cdot \text{N}=\text{N}-\text{C}_6\text{H}_4-\text{N}=\text{N}-\text{C}_6\text{H}_4-\text{N}_4\text{O}_5\text{S} \cdot \text{HgCl}_2 \end{array}$
Ortho-Oxy-Benzylidene Amino Phenyl-para Mercuric Acetate.....	44.0	$\begin{array}{c} \text{OH} \quad \text{H} \\   \quad   \\ \text{C}_6\text{H}_4-\text{C}=\text{N}-\text{C}_6\text{H}_4-\text{O}-\text{C}(=\text{O})-\text{CH}_3 \end{array}$
1-Amino-2 [para-Naphthalin-Azophenyl Mercuric Acetate] 5 Sulphonic Acid .....	34.2	$\begin{array}{c} \text{NH}_2 \quad \quad \quad \text{O} \\   \quad \quad \quad    \\ \text{C}_6\text{H}_4-\text{N}=\text{N}-\text{C}_{10}\text{H}_6-\text{C}(=\text{O})-\text{O}-\text{C}(=\text{O})-\text{CH}_3 \end{array}$
Methylene Blue-Mercuric Chloride...	28.94	$\begin{array}{c} \text{N}=\text{N} \\   \quad   \\ \text{C}_6\text{H}_4-\text{S}-\text{N}(\text{CH}_3)_2 \end{array} \cdot \text{HgCl}_2$
Iod-Methylene Blue Mercuric Chloride .....	24.75	$\begin{array}{c} \text{N}=\text{N} \\   \quad   \\ \text{C}_6\text{H}_4-\text{S}-\text{N}(\text{CH}_3)_2 \end{array} \cdot \text{HgCl}_2$
Methylene Green Mercuric Chloride..	31.6	$\begin{array}{c} \text{N}=\text{N} \\   \quad   \\ \text{C}_6\text{H}_4-\text{S}-\text{N}(\text{CH}_3)_2 \end{array} \cdot \text{HgCl}_2$
Diazo-Amino Methylene Blue ortho-Toluidin di Mercuric Chloride....	29.7	$\begin{array}{c} \text{N}=\text{N} \\   \quad   \\ \text{C}_6\text{H}_4-\text{S}-\text{N}(\text{CH}_3)_2 \end{array} \cdot \text{HgCl}_2$
Diazo-Amino Methylene Blue Brom Hydrate ortho-Toluidin Mercuric Bromide .....	39.5	$\begin{array}{c} \text{N}=\text{N} \\   \quad   \\ \text{C}_6\text{H}_4-\text{S}-\text{N}(\text{CH}_3)_2 \end{array} \cdot \text{HgBr}$
Diazo-Amino Methylene Blue ortho Phenol Mercuric Chloride.....	29.7	$\begin{array}{c} \text{N}=\text{N} \\   \quad   \\ \text{C}_6\text{H}_4-\text{S}-\text{N}(\text{CH}_3)_2 \end{array} \cdot \text{HgCl}_2$

amount of such insoluble compounds was weighed into each tube of melted agar. The tubes were well shaken and cooled quickly in ice water so that time was not given for the drug to settle out. In this way a fine suspension of the drug in agar was used, and, as can be seen from table 2, such fine suspensions showed quite as great bacteriostatic power as did the true solutions. It is probable that this is due to a slight unrecognizable solubility, so that in a dilution of 1:20,000 the compounds were really in solution. Only in the case of iodo-mercuro-hemol did even this method fail to give good results. When the hot melted agar was added to the powdered mercuro-iodo-hemol, it formed large, coarse, flocculent masses, which would not break up again. This probably explains the fact that only a dilution of 1:1,000 completely inhibited while a dilution of 1:5,000 partially inhibited growth and that there was good growth on all the higher dilutions. Hence, it required 0.12 mg. of mercury in the form of mercuro-iodo-hemol per c c of agar for complete inhibition of growth, far the largest amount required in any of the tests. Mercuric chloride stood next in the amount of mercury per c c needed for inhibition. This seemed surprising, as mercuric chloride is usually considered high in bacteriostatic power. However, I found it impossible to prepare my agar tubes of mercuric chloride without a certain amount of precipitate in the bottom of the tubes. This consisted of metallic mercury, and, of course, considerably reduced the percentage of mercuric chloride in the agar. However, Schamberg and his co-workers<sup>1</sup> also found that many of their organic mercury compounds had a much higher germicidal power than did mercuric chloride, and in their tests the dilutions were made in distilled water. The calculations of the amount of mercury per c c were made in order to determine whether the inhibitory action on the growth of the tubercle bacillus depends solely on the proportionate amount of pure mercury in the molecule, as Schamberg<sup>8</sup> found was true of the toxicity, or whether, as Schamberg<sup>1</sup> found in his bactericidal studies with *B. typhosus* and *Staph. aureus*, "the antiseptic value is not related to the amount of pure mercury in the substance, but rather to the chemical constitution of the molecule." By examining the last column of table 2, it may be seen that 4 compounds are, in proportion to the mercury content, considerably higher than the others in bacteriostatic power. These are in order: (1) allyl alcohol mercuric acetate, (2) double salt of methylene blue and mercuric chloride, (3) fluorescein and mercuric chloride and (4) 1-amino, 2 (paranaphthalin azophenyl mercuric acetate) 5 sulphonic acid. The second and

third are double salts of mercuric chloride and a dye which alone has considerable inhibitory power. This is especially true of methylene blue. In the first, mercury is bound to a carbon of an alcohol ring, while in number 4, mercury is bound by one bond to a carbon of a phenyl ring. The double salt of the dye trypan blue and mercuric chloride requires over twice as much mercury per c c to inhibit as the double salt of methylene blue and mercuric chloride, but the dye trypan blue has itself practically no inhibitory power. It seems necessary to conclude from these findings that the different constituents of the molecule play their own rôle in the bacteriostatic action of the mercury compounds. The question of the relation of the position of the mercury in the molecule to bacteriostatic and bactericidal action will be discussed in another paper.

TABLE 2  
BACTERIOSTATIC POWER

Mercurial	Soluble in	Growth in Tubes								Mg. Hg per C c Needed to Inhibit
		1: 1000	1: 5000	1: 10,000	1: 20,000	1: 50,000	1: 100,000	1: 500,000	Con- trol	
Mercuric chloride	Water	None	None	None	None	Good	Good	Good	Good	0.093
Mercury sulpho- cyanide	Insoluble, good sus- pension	None	None	None	None	Good	Good	Good	Good	0.031
Mercury potas- sium cyanide	Water	None	None	None	None	Good	Good	Good	Good	0.034
Allyl alcohol mer- curic acetate	Dilute alkali	None	None	None	None	None	Very slight	Slight	Good	0.012
Mercuric succini- mide	Water	None	None	None	None	Good	Good	Good	Good	0.025
Mercuric salicy- late	Insoluble, good sus- pension	None	None	None	None	Good	Good	Good	Good	0.021
Mercurio-iodo- hemol	Insoluble, poor sus- pension	None	Slight	Good	Good	Good	Good	Good	Good	0.12
Mercuriol (mercu- ric nucleinate)	Water	None	None	Slight	Good	Good	Good	Good	Good	0.02
Mercury phenol- phthalein	Dilute alkali	None	None	None	Good	Good	Good	Good	Good	0.039
Fluorescein mer- curic chloride	Dilute alkali	None	None	None	None	Good	Good	Good	Good	0.016
Ortho-oxyben- zylidene amino phenyl p a r a mercuric acetate	Dilute alkali	None	None	None	None	Good	Good	Good	Good	0.022
1-amino-2(p-naph- th a l i n azo- phenyl mercuric acetate) - 5 sul- phonic acid	Dilute alkali	None	None	None	None	Slight	Slight	Very slight	Good	0.017
Trypan blue mer- curic chloride	Slightly in hot water	None	None	Good	Good	Good	Good	Good	Good	0.03
Methylene blue mercuric chloride	Insoluble, good sus- pension	None	None	None	None	Slight	Good	Good	Good	0.014
I o d methylene blue mercuric chloride	Insoluble, good sus- pension	None	None	None	Very slight	Good	Good	Good	Good	0.024

It will be seen that all the compounds showed some inhibitory power, that nearly all inhibited completely at 1:10,000, while many also inhibited at 1:20,000.

With only 3 compounds, allyl alcohol mercuric acetate, double salt of methylene blue and mercuric chloride, and 1-amino, 2 (p-naphthalin azo-phenyl mercuric acetate) 5 sulphonic acid, did the bacteriostatic action go farther than 1:20,000.

The bactericidal power of the compounds used in these experiments has not been tested, partly because of the insolubility or difficult solubility of most of the preparations. In most of the experiments with antiseptics and drugs used in my former experiments, we have found that a somewhat lower concentration was required for inhibition than for complete bactericidal action. In a report by DeWitt and Sherman,<sup>12</sup> we found that human tubercle bacilli distributed in a thin layer on garnets and exposed for 24 hours to mercuric chloride in a dilution of 1 part of the drug to 100,000 parts of water were killed so that they failed to infect guinea-pigs.

It required 1 part in 50,000 to kill them in clumps in 1 hour so that they would not grow on agar tubes and 1 part in 1,000 of water to kill them in 1 hour so that they would not cause the disease in animals. In all cases, the garnets, after exposure to the mercuric chloride solutions, were well washed with ammonium sulphide solution to neutralize the mercury and then with 4 wash waters, so that none or very little of the drug could have been carried over to continue its action. It is difficult to explain this apparently much higher bactericidal than bacteriostatic power of mercuric chloride except on the assumption that a considerable part of the mercury must have been thrown out of solution in the agar. However, Lewis<sup>13</sup> concludes that there is no close or constant correlation between the bactericidal and inhibitory action of chemicals.

#### THERAPEUTIC EXPERIMENTS

##### 1. *Mercurous Chloride*

Six guinea-pigs were inoculated subcutaneously with 0.2 c.c. of a suspension of human tubercle bacilli of the strain known as "Old Human" to distinguish it from the various strains acquired more recently. The suspension was diluted to the point at which it was just slightly opalescent. These 6 animals were each fed daily one pill containing 1 mg. of mercurous chloride. One pig died in 2 days, before any tuberculous involvement was possible. The others lived

<sup>12</sup> J. Infect. Dis., 1914, 15, p. 245.

<sup>13</sup> J. Exper. Med., 1917, 25, p. 441.

167, 231, 240, 245, and 325 days, an average of 241 days, and all showed marked tuberculous involvement of lymph glands, spleen, liver and lungs. They had received by mouth 125, 198, 209, 213, and 282 mg., an average of 205 mg. Control pigs were inoculated on the same day with the same dose of the same suspension and lived 85, 183, 193, 193, and 193 days, an average of 169 days. All the control animals also showed marked generalized tuberculosis. The only difference, then, between the treated and the untreated animals was that the treated animals lived longer than the untreated, but the extent of the disease at death was about the same.

### 2. *Mercuric Sulphocyanide*

Six guinea-pigs were inoculated with 0.2 c.c dilute suspension of the strain known as "Old Human." They received one subcutaneous injection of 0.5 mg. and one of 1 mg. and were fed 1 mg. of the drug per day. The compound was toxic enough so that daily feedings had to be interrupted at intervals for a week or more and then resumed. Two of the animals received one intracardiac injection of 1 mg. One of these died the next day and the other 33 days later. The length of life after inoculation was 42, 85, 96, 143 and 193 days. The average length of life was 112 days. The amount of the drug administered was 39.5, 60, 93, 124, 166 mg., or an average per animal of 91.5 mg. All the animals had enlarged caseous inguinal glands. The one dying in 42 days had no other tubercles visible macroscopically. The others showed some tubercles in the organs but only in the one living 193 days was the disease marked.

The duration of life of the untreated controls was 98, 111, 116, 117, 143 and 156 days, or an average of 123.5 days. Hence with this treatment, the treated animals died more quickly than the untreated controls. On the other hand, the extent of the disease in all the controls was much greater than in the treated animals, the liver, spleen and lungs in those dying after 98, 111, 116, and 117 days being more involved than in the treated animals living 143, and 193 days. This suggests that the drug had a certain slight inhibitory action over the progress of the disease although not enough to prevent it completely, while its toxic action was sufficient to shorten life in many of the animals.

### 3. *Mercury Potassium Cyanide*

Six guinea-pigs were inoculated with 0.2 c.c of dilute emulsion of "Old Human" tubercle bacilli. Each received one subcutaneous, one intracardiac and one intramuscular injection and was fed 1 mg. per day. One died on the eighth day after inoculation with no sign of tuberculosis. One died on the thirty-second day showing an enlarged regional gland and a few small tubercles in the spleen. The others lived 86, 162, 169 and 238 days, or an average of 166 days. All showed marked tuberculosis of lymph glands, spleen, liver and lungs. The controls of this series lived 98, 111, 116, 117, 143 and 156 days, or an average of 123.5 days, and all had a marked generalized tuberculosis. In this series, if we leave out of consideration 2 treated animals that died early from some cause other than tuberculosis, the treated animals lived longer than the controls, but the extent of the disease was about the same in the two sets.

#### 4. *Allyl Alcohol Mercuric Acetate*

This preparation was made for me in the chemical laboratory of the University of Chicago. My reason for having it made was the reputation that allyl alcohol and some of its derivatives have acquired in the clinical treatment of tuberculosis. As is seen in table 2, this mercurial compound of allyl alcohol has a high bacteriostatic power over the growth of the human tubercle bacillus. The compound could be dissolved in water to make a solution of one part in 1,000. Six guinea-pigs were inoculated with 0.05 mg. of culture strain 1305, isolated by Dr. H. J. Corper at the Chicago Municipal Sanatorium about 5 years ago. Each animal received a subcutaneous injection of 1 mg. of the allyl alcohol mercuric acetate in 1 cc of water. Then each was fed 1 mg. pill per day up to the time of death. These animals lived 46, 56, 99, 102, 104 and 140 days, or an average of 91 days. They had received 41, 49, 87, 87, 88 and 118 mg. of the drug, or an average of 78 mg. for each animal. All showed marked tuberculosis of lymph glands, spleen, liver and lungs. The controls of this series lived 77, 78, 79, 84, 119 and 119 days, or an average of 92.6 days. All the animals showed marked generalized tuberculosis. In spite then, of the high bacteriostatic power of allyl alcohol mercuric acetate in vitro, it seems to have no therapeutic effect on animals inoculated with a virulent strain of tubercle bacilli, since both length of life and extent of disease are practically the same in the treated and untreated animals. It is probable that this drug fails to penetrate the young tubercles, or is broken up and loses its antiseptic power before it reaches them.

#### 5. *Mercuric Succinimide*

This is the mercury compound which, perhaps because of its being a soluble organic mercury compound, has been especially used in the clinical treatment of tuberculosis. It was used largely by B. L. Wright and his followers, although some of them substituted mercuric chloride for the succinimide. Six guinea-pigs were inoculated with 0.2 cc of the dilute suspension of the "Old Human" strain of tubercle bacilli. Subcutaneous and intracardiac injections were used at first, then pills containing 1 mg. of the drug were fed daily. One lived only 10 days, succumbing on the day following an intracardiac injection and, of course, showed no tuberculosis. The others lived 85, 95, 154, 189 and 219 days, an average of 148 days. They received 84, 90, 154, 214 and 240 mg. of the drug, or an average of 156 mg. The extent of the disease was distinctly limited in this series. The pig that died in 85 days showed only an involvement of the regional lymph glands with necrotic masses in the spleen. Even the one that lived the longest showed but slight involvement of the spleen and lungs and regional lymph glands. Some had partial necrosis of the liver and some showed cirrhosis of the liver, but the involvement of the organs was slight for the duration of the disease. The controls lived 98, 111, 116, 117, 143 and 156 days, or an average of 120 days. All had marked tuberculosis of lymph glands, spleen, liver and lungs. The treated animals have lived on the average somewhat longer than the controls, although 2 died earlier and 2 lived much longer than any of the controls. The extent of the disease in the treated was considerably less than in the untreated animals.

#### 6. *Mercuric Salicylate*

Five guinea-pigs were inoculated with 0.2 c.c. of dilute suspension of Miller's strain of human tubercle bacilli, a moderately virulent strain. Since mercuric salicylate is insoluble, a suspension was made in sterile paraffin oil and an amount containing 2 or 3 mg. of the drug injected intramuscularly once a week. On the other days of the week, a pill, containing at first 1 mg. and afterward 2 mg., was fed to each animal. This dosage was evidently too toxic, as the animals lived only 13, 27, 27, 37 and 59 days, having received 12, 24, 24, 35 and 67 mg. of the drug. The inguinal glands were enlarged and caseous in all except the one dying in 13 days, and the spleen was involved in some of the others. Hence, even in these doses which so quickly killed the animals, there was evident little, if any, influence on the progress of the infection. The controls of this series lived 91, 96, 97, 104, 121 and 226 days, an average of 122.5 days, and all showed marked generalized tuberculosis.

#### 7. *Mercurio-Iodo-Hemol*

This is an insoluble commercial preparation obtained from Merck and Company. Hemol is a hemoglobin preparation, and the compound used is said to contain 12.35% of mercury and 28.6% of iodine. From 2-5 grains by mouth three times per day is the dose recommended for syphilitic patients. Six guinea-pigs were inoculated with 0.2 c.c. of a dilute suspension of the "Old Human" strain of tubercle bacilli. Each animal was fed daily a pill containing 1 mg. of the mercurio-iodo-hemol preparation and received several subcutaneous injections of a suspension in gelatin and an intramuscular injection. They lived 67, 96, 110, 133, 135 and 171 days, or an average of 120 days, and received 41, 86, 97, 109, 119 and 147 mg. of the drug, an average of 99½ mg. All showed considerable tuberculous involvement of the lymph glands, liver, spleen and lungs. The controls of this series lived 98, 111, 116, 117, 143 and 156 days, or an average of 123.5 days, and all showed an extreme degree of generalized tuberculosis. Hence, the hemol mercury preparation, while not especially toxic, seems to have had no beneficial influence on the progress of the disease.

#### 8. *Mercuriol*

Mercuriol or mercury nucleide is also a commercial preparation obtained from Merck and Company. It is soluble in water and is said to contain 10% of mercury. It was tested in this series of experiments, especially because of the favorable reputation which nuclein and nucleinic acid have long borne in the clinical treatment of pulmonary tuberculosis. The mercuriol is freely soluble and neither very toxic nor locally irritating, so that injections of various kinds could be continued much longer than with most of the mercurial preparations. Several sets of animals were therefore tried out in testing the therapeutic properties of this drug.

(a) Six guinea-pigs were inoculated with 0.2 c.c. of a dilute suspension of "Old Human" tubercle bacilli. These were fed daily pills containing each 1 mg. of the drug, the daily dosage being increased according to the condition of the animal. They also received injections once a week, 2 subcutaneous injections of 1 and 3 mg. and 4 intracardiac injections of 2, 3, 4 and 5 mg. of the drug. They lived 33, 42, 47, 56, 82 and 234 days, or an average of 82 days. They received 36, 49, 57, 76, 129 and 349 mg., or an average of 116 mg. of the drug. Three of these animals died as a direct result of an intracardiac injection, one died from a septic abortion, while none had

any considerable tuberculous involvement more than enlarged and partly caseous regional lymph glands. The controls of this set lived 26, 56, 71, 137, 202 and 217 days, or an average of 118 days, and all except the first two to die had marked generalized tuberculosis.

(b) The second set consisted of 6 guinea-pigs inoculated with the same dose of the same strain as set 1. They were treated merely by feeding mercuriol pills, gradually increasing the dose as the condition of the animal permitted. Two other guinea-pigs were inoculated with the caseous substance from a lymph gland taken from a pig of set 1 and were treated with the pigs of set 2, after a local tubercle developed. These lived 48, 90, 122, 139, 160, 271, 271, 271 days, or an average of 171 days. They received by mouth 42, 86, 166, 199, 186, 186, and 186 mg. In all these animals there were found enlarged caseous glands in the inguinal region and from slight to moderate involvement of the liver, spleen and lungs. In none of the animals of this set were these organs normal or even completely free from tubercles, as they were in set 1. The controls of this set lived 85, 183, 193, 193 and 193 days, or an average of 169 days, a slightly lower average length of life than in the treated animals because 3 of the treated animals lived much longer than the others and much longer than any of the controls. All of the controls, except the one dying on the eighty-fifth day, exhibited much more marked general tuberculosis than did the treated animals.

(c) The third set was inoculated with the same dose of the same strain as the other two sets. It was treated by feeding and intraperitoneal injections. These died in 47, 78, 118, 159 and 164 days, or an average of 113 days. They had received 52, 78, 126, 142 and 155 mg., or an average of 111 mg. of the drug. The one dying after 47 days had enlarged caseous glands in both groins and a few in the spleen and liver. The one dying in 78 days had no caseous glands and no macroscopic tubercles in the liver, spleen or lungs. The 3d, 4th and 5th to die showed no tubercles in any of the organs and no caseous glands. This pathologic condition would have seemed somewhat encouraging for the claims of mercuriol had it not been that the control animals of that set lived much longer than the treated and exhibited about the same degree of tuberculous involvement. This seemed to indicate that the "Old Human" strain was losing its virulence to a degree which made it hardly usable for therapeutic experiments. In connection with set 3, a number of uninoculated guinea-pigs were treated with mercuriol in the same way as the infected pigs. These pigs were killed after the tuberculous pigs had died. No pathologic changes were noted, except hemorrhages and hemorrhagic exudates in most of the animals.

In regard to mercuriol, we must conclude that, while it is quite nontoxic and nonirritating and seems to have some slight inhibiting influence over the spread of the disease in the animal, that influence is not sufficient to encourage us in further experiments, since in all probability, with more virulent strains, the influence of the drug would either not be felt or, as often happens, would stimulate the organism to increased growth and virulence.

#### 9. Mercury Phenolphthalein

This preparation was made for me in the chemical laboratory of the University of Chicago according to suggestions made by Hahn and Kostenbader.<sup>14</sup> It is insoluble in water but dissolves in alkalis. The mercury makes up about 39% of the molecular weight. (See table 1.)

<sup>14</sup> Ztschr. f. Chemotherapie, O., 2, O, p. 71.

Six guinea-pigs were inoculated with 0.2 cc of a dilute suspension of "Old Human" strain of tubercle bacilli and each was fed daily first one and later two pills containing each 2.6 mg. Three of the 6 animals died during the first month of some cage infection with no sign of tuberculosis. The other three lived 210, 244 and 306 days and, at death, showed advanced tuberculous involvement of lymph glands, liver, spleen and lungs. The controls died after 126, 170, 182, 187, 192 and 205 days, all showing marked general tuberculosis.

#### 10. *Mercury Tetraiod Phenolphthalein*

This was also suggested by Hahn and Kostenbader and, having 4 iodine atoms in the molecule given for mercury phenolphthalein, was also tested, the animals being inoculated and then fed daily. Aside from 2 animals that died early, they lived 133, 151, 166 and 241 days, and all showed at death advanced tuberculosis of lymph glands, spleen, liver and lungs. The controls lived 122, 133, 150, 169, 176 and 295 days. All had extensive advanced tuberculosis. Hence these 2 phenolphthalein dyes seem to have no influence on the extent and distribution of the disease.

#### 11. *Double Salt of Fluorescein and Mercuric Chloride*

This also is one of Hahn and Kostenbader's "mercurialized dyes." Six guinea-pigs were inoculated with 0.2 cc of a dilute suspension of the "Old Human" strain and then fed with the dye. Three died during the first month of cage infections. The other three lived 58, 88 and 217 days having received 250, 426 and 950 mg. of the dye. The one living 58 days showed no generalized tuberculosis, the second contained tubercles only in the lungs, while the third had only a few small tubercles in the lungs, but the liver and spleen were full of tubercles. The controls lived 126, 170, 182, 187, 192 and 205 days, or an average of 177 days, and all showed extensive advanced general tuberculosis.

In spite of the fact that the involvement in the controls was much greater than in the treated pigs, we cannot under these conditions consider that the difference was due to the treatment, since all animals that lived as long as did the controls had some generalized tuberculosis, even though not distributed through all the organs.

#### 12. *Double Salts of Trypan Blue and Mercurous Chloride and Mercuric Chloride*

These salts were made for me by Dr. Walter Fraenkel, and the method of making was described in a previous paper.<sup>15</sup> At that time it was found that these salts had some tuberculocidal power since 0.1 cc of a suspension of tubercle bacilli exposed for 24 hours to 1% solution of the salts and then injected into guinea-pigs failed in most cases to cause disease. The inhibiting action of the mercuric salt was surprisingly low, since only the 1:1,000 and 1:5,000 dilutions caused complete inhibition. Six guinea-pigs were inoculated with 0.2 cc of dilute suspension of the "Old Human" strain. They received the mercurous salt of the dye both by daily feeding of 1, 2 or 3 mg. and by occasional intramuscular injections of a suspension in oil. They lived 33, 35, 38, 73, 93 and 219 days, or an average of 81 days. They had received

<sup>15</sup> Jour. Infect. Dis., 1914, 14, p. 498.

37, 43, 52, 97, 181 and 192 mg., or an average of 100 mg. All showed generalized tuberculosis in moderate degree. A second set was inoculated with 0.1 cc of the Miller's, a more virulent strain. These were both fed and injected. They lived 91, 94, 137 and 139 days, or an average of 115 days. They received 110, 112, 137 and 139 mg. of the dye. All showed an advanced generalized tuberculosis. Hence, these mercury salts of trypan blue have only slight bactericidal and inhibitory power and no therapeutic influence over the disease.

### *13. Mercury Derivations of Aromatic Amines*

Jacobs and Heidelberger<sup>16</sup> described some mercury derivatives of aromatic amines. As some of these preparations looked as if they might be of interest in my work, I had my chemist make two of them according to their directions.

(a) 4-ortho-oxybenzylidene amino phenyl para mercuric acetate, which contains 44.02% of mercury. This compound is soluble in dilute alkali. Six guinea-pigs were inoculated with 0.2 cc of a dilute suspension of "Old Human" strain of tubercle bacilli. One mg. pills were fed to each pig daily and several injections were given. Two of the pigs died shortly after an intracardiac injection. The other animals lived 33, 68, 78 and 220 days, or an average of 99½ days. They had received 32, 40, 53 and 151 mg., or an average of 69 mg. per animal. The three that died earliest had no or slight tuberculosis, but the one living 220 days showed an extensive generalized tuberculosis. The controls lived 24, 119, 184, 238, 264 and 315 days, or an average of 207 days, much longer than the treated animals and all except the first showed marked and extensive generalized tuberculosis.

(b) 1-amino-2(para-naphthalin azophenyl mercuric acetate) 5-sulphonic acid. This contained 34.24% of mercury and was also soluble in dilute alkali. The inhibitory power was high, since there was complete inhibition up to 1:20,000 and partial inhibition even to 1:500,000. Two sets of animals were used for this compound. The first set was inoculated and treated in the same way as the set just described. These lived 10, 30, 50, 51, 65 and 250 days. The first ones died from the effect of an injection. Only the one that died last after 250 days showed tuberculosis. The second set was inoculated with the same dose of the same strain and treated mostly by feeding, only one subcutaneous injection having been given. These animals lived 36, 118, 146, 155, 188 and 255 days, an average of 149 days. All showed slight to moderate generalized tuberculosis with considerable tendency to the fibrous form of tuberculosis. The controls lived 42, 168, 199, 219, 276 and 281 days, or an average of 197 days, much longer than the treated pigs, and all except the first showed an advanced generalized tuberculosis.

### *14. Mercury Compounds of Methylene Blue and Its Derivatives*

This group of compounds has been of especial interest to me because of the position methylene blue itself has occupied as an internal germicide and also in the treatment of tuberculosis. Some of the work has already been reported briefly.<sup>17</sup> In this present report, the work of the various mercuric compounds of the group of dyes related to and derived from methylene blue will be reported more in detail. In the earlier work and by different methods than those

<sup>16</sup> J. Biol. Chem., 1914, 20, p. 513.

<sup>17</sup> DeWitt: Jour. Infect. Dis., 1913, 13, p. 178; Transactions of the Twelfth Annual Meeting of the National Tuberculosis Association, 1916.

used, I found the methylene blue chloride caused complete inhibition of the human tubercle bacillus in a dilution of 1:5,000, while Paul Lewis<sup>18</sup> by still another method found that 1:1,000,000 was the highest concentration at which the growth equaled that on control flasks. Methylene blue was also shown by me to have some bactericidal action on the tubercle bacillus.

(a) The double salt of methylene blue and mercuric chloride, which was made by my chemical assistants by adding a solution of mercuric chloride to a solution of methylene blue and thoroughly washing and drying the precipitate thus formed, contains about 34% mercury and is quite insoluble, but forms a good suspension on shaking in hot agar and then cooling and slanting quickly. There was no growth in tubes up to 1:20,000 and only slight growth in the tubes containing a dilution of 1:50,000. The amount of mercury per c.c. in the highest dilution which completely inhibited was 0.014, which was less than completely inhibited in any other mercurial tested, except the allyl alcohol mercuric acetate. In the first experiment with this drug, 4 of the 6 guinea-pigs died within the first 3 weeks. The other 2 lived 130 and 208 days. Each pig was fed 1-2 mg. every day during life and received injections once a week. There was practically no tuberculous involvement even in the animal that lived 208 days. Another set of animals was therefore inoculated in the same way with 0.2 c.c. of dilute suspension of the "Old Human" strain of tubercle bacilli. These received no injections but were fed daily pills containing a dose of 1-2 mg. These pigs lived 147, 237, 265, 269 and 288 days, an average of 241 days. They received by mouth during life 264.6, 460, 460, 500 and 520 mg. of the drug, or an average of 440.9 mg. per animal. Four of these pigs showed at necropsy only slight involvement of regional lymph glands and of the spleen; but the fifth showed extensive and advanced tuberculosis of lymph glands, spleen, liver and lungs. The controls of this series lived 126, 170, 182, 187, 192 and 205 days, an average of 177 days, a much shorter time than the treated animals lived, while every one of the controls showed marked, extensive and advanced tuberculosis of lymph glands, spleen, liver and lungs. These results were so good that it seemed well worth while to carry the experiment further, and another set of guinea-pigs was inoculated with the same dose of the same strain of human tubercle bacilli. These also were fed daily and no injections were given. These lived 96, 188, 247, 267, 298 and 298 days, an average of 231 days. In 4 of these the tuberculous involvement was slight, while in 2 it was extensive. The controls of this series lived 42, 168, 199, 219, 276 and 281 days, an average of 197 days, and all except the first showed as extensive tuberculosis as the worst two of the treated animals. The effect of treatment in this set seemed much less than in the other sets described, but the treated animals lived longer and showed less extensive disease than the untreated.

The next set consisted of 5 guinea-pigs inoculated with 0.2 c.c. dilute suspension of the "Old Human" strain. They received the drug by daily feeding of 1-2 mg. pills and intramuscular injection of the drug in cotton seed oil. They lived 100, 147, 265, 335 and 733 days. None of these animals had any definite tubercles in any of the internal organs, though all had slightly enlarged noncaseous glands in the inguinal region. The controls of this set lived 145, 172, 222, 232 and 266 days, an average of 207 days, and all had advanced tuberculosis of lymph glands, liver, spleen and lungs. The next set consisted

<sup>18</sup> Jour. Exper. Med., 1917, 25, p. 441.

of 6 guinea-pigs inoculated with the same dose of the same strain as the other sets and treated by daily feedings and weekly intramuscular injections of the double salt in cotton seed oil. They lived 97, 101, 103, 161, 402, 813 and 1,000 days. The 2 that lived over 2 years showed no sign of tuberculosis except slightly enlarged and hard glands in the groins, which showed necrotic fibrous encapsulated tubercles. The others showed no sign of tuberculosis, except some enlarged, noncaseous glands. This would have been satisfactory had it not been that the controls of this set, although they died much earlier than some of the treated animals, living 110, 160, 204, 233 and 234 days, and although they had enlarged and caseous glands, showed but slight or moderate tuberculosis of the internal organs. It seemed safe to conclude from all these experiments that animals inoculated with a strain of low virulence certainly received considerable benefit from treatment with small doses of the double salt of methylene blue and mercuric chloride. A much more virulent strain was then used to determine whether the effect observed in the animals inoculated with the strain of low virulence would also be observed in those inoculated with a strain of high virulence. The strain used was called "1305" and was one isolated in about 1916 by Dr. Corper from sputum obtained from a patient in the Chicago Municipal Tuberculosis Sanatorium. Eight guinea-pigs were inoculated subcutaneously, each receiving 0.05 mg. of the culture. They were treated by daily feeding of pills containing each 1 mg. of the double salt of methylene blue and mercuric chloride. They lived 54, 58, 70, 76, 77, 111, 121 and 122 days, or an average of 73 days. They had received 42, 50, 60, 65, 66, 98, 104 and 105 mg. of the drug, or an average of 74 mg. per animal. All the animals showed some tuberculosis of the internal organs at necropsy and in nearly all the disease was moderately or extremely extensive. The controls lived 60, 109, 111, 129 and 172 days, or an average of 116 days, and all showed marked tuberculosis of the lymph glands, spleen, liver and lungs. In this set there was practically no difference between the treated and untreated guinea-pigs. Another set was inoculated with 0.05 mg. of 1305, then fed 1 mg. of the double salt daily, as in the last set, but these also received several subcutaneous injections of 1 mg. of egg albumin. These lived 92, 104, 106, 121, 124 and 214 days, and nearly all showed extensive involvement of lymph glands, liver, spleen and lungs. Still another set, inoculated in the same way with the same dose of the same strain as the set described above and as the controls given under the first of these three 1305 sets, received in addition to the feeding with the double salt of methylene blue and mercuric chloride several subcutaneous injections of a vaccine made by heating a suspension of the same strain with which they were inoculated to 60 C. for one hour. These lived 30, 67, 85, 111, 130, 143, 143, 153 and 156 days, or an average of 113 days as compared with an average of 116 days life of the controls. All of the guinea-pigs of this set also showed extensive tuberculosis of the internal organs in a degree that is apparently about equal to that of the control animals.

Another set was inoculated each with 0.05 mg. of the virulent strain 1305 and then fed daily from 1 to 2 mg. of the double salt. They lived 62, 73, 95, 96, 101, 129, 164 and 259 days. They had received by mouth before death 63, 73, 92, 93, 97, 121, 151 and 389 mg. Five of these 8 guinea-pigs showed either no or slight tuberculous involvement, having died from some acute infection. The 2 that died after 96, 101 and 259 days showed a rather extensive general involvement. Six of the 8 controls of this set have died after

14, 66, 66, 207, 213 and 267 days. The one dying after 14 days showed no tuberculosis, and one of those dying after 66 days had a slight degree of the disease; in the other 4 the glands, liver, spleen and lungs were extensively involved.

Hence, we must say that, as far as we may conclude from these experiments, the double salt of methylene blue and mercuric chloride, if fed in 1 mg. doses, exerts no or but little beneficial influence on the rapidly progressive type of tuberculosis caused in guinea-pigs by inoculation with a virulent human strain, although in the type caused by inoculation with a strain of low virulence, it seems to exert a distinct and considerable influence, although it cannot be said to cure, even in these cases. If we may judge by the length of life and by the condition of the animals, we cannot consider that it had in any case an injurious influence if given by mouth and, if given by intramuscular injection, there seems to have been no other ill effect than the local infiltrations and occasional ulcerations usually caused by any oil emulsion.

In addition to the experiments described on the use of the double salt of methylene blue and mercuric chloride, 2 sets of animals were treated with alternate injections of methylene blue and of calomel and one set using alternate injections of methylene blue and of mercuric chloride. This was done with the idea that a compound of methylene blue and mercury might thus be formed internally and perhaps be more efficacious. The first set treated with methylene blue and calomel died during the first month, showing no sign of tuberculosis. The second set received the methylene blue by intracardiac injection and the mercurous chloride by intramuscular injection, but both in smaller individual doses than were used in the first set of animals. Three of the animals of this set died after the first intracardiac injection, 2 from abortion and the others from the heart injection. The other 3 received the injections about once a week for one month and afterward no further treatment and lived 172, 211 and 312 days. Only the last one of these showed any considerable tuberculous involvement. The controls of this set lived 26, 56, 71, 137, 202 and 217 days. The strain used for inoculation in this set was the "Old Human" which had by this time partly lost its virulence, so that only the controls that had lived 200 or more days showed extensive tuberculosis, while in the treated animals, only the one that lived 312 days showed any considerable disease.

The next set were inoculated subcutaneously with 0.2 c.c. of "Old Human" strain of tubercle bacilli and for a little over one month received weekly intraperitoneal injections of methylene blue and mercuric chloride separately. These drugs were also fed to them on alternate days. They lived 41, 65, 106, 106, 110 and 247 days, or an average of 113 days. Those that lived 106 and 110 days showed moderate generalized tuberculosis, but the others, even the one living 247 days, showed none. The controls of this set lived 110, 160, 204, and 233 and 234 days and showed little generalized tuberculosis. All showed enlarged and partly caseous glands. Hence this treatment of methylene blue and mercuric chloride given separately cannot be said to have had any beneficial effect even in the animals inoculated with a strain of human tubercle bacilli of extremely low virulence.

(b) Double salt of iod methylene and mercuric chloride. Iod methylene blue was made by my chemical assistant and was found to inhibit growth of tubercle bacilli in dilutions of 1:5,000, just as did methylene blue itself. It

is probably the same compound recommended by von Linden. The double salt was made by adding a solution of mercuric chloride to a solution of iod-methylene blue and washing the precipitate thus formed. It contains 24% of mercury and its inhibiting power was a little less than that of the double salt of methylene blue and mercury, since there was slight growth in the tubes containing the dye in a dilution of 1:20,000, about corresponding to the growth in the tubes containing dilutions of 1:50,000 of methylene blue and mercuric chloride. Six guinea-pigs were inoculated with 0.2 c.c. of a dilute suspension of "Old Human" strain of tubercle bacilli and then fed the dye daily in 1 mg. doses and injected once a week subcutaneously during the first month. They lived 81, 142, 143, 165, 204 and 453 days, an average of 198 days, and had received 89, 156, 157, 178, 224 and 509 mg. of the dye, or an average of 219 mg. per animal. All the animals of this set exhibited extensive tuberculosis. The controls lived 122, 147, 176, 197, 215 and 238 days, or an average of 182.5 days. The average duration of life of the controls was less than that of the treated animals, but that was due to the one animal of the treated set which lived more than twice as long as any of the others and almost twice as long as the last of the controls to die. The extent of the disease in the controls was about the same as in the treated animals, so that the double salt of iod-methylene blue and mercuric chloride seems to have had in this experiment no beneficial action, although the strain with which the animals were inoculated was one of fairly low virulence. This may partly confirm a statement made by Lenard<sup>19</sup> that iodine prevents the deposition of the mercury in the liver, which is said by Blumenthal and Oppenheim<sup>20</sup> to have something to do with the therapeutic power.

(c) Double salt of methylene green and mercuric chloride. Methylene green has one nitro group in the methylene blue molecule. The dye itself has not in my experiments shown itself equal to the methylene blue in inhibitory, bactericidal or therapeutic power. The mercury preparation has been made by my assistants by the same method as the other double salts. It is quite insoluble. Six guinea-pigs inoculated with 0.2 c.c. of a dilute suspension of "Old Human" strain were fed daily and received several injections. Four of the 6 died within the first month, the other 2 living 201 and 238 days and showing extensive and advanced tuberculosis. Therefore another set was inoculated in the same way and received no injections, but simply daily feedings. These lived 73, 199, 220, 226 and 378 days, an average of 219 days. They had received 114, 398, 413, 450 and 648 mg., or an average of 405 mg. of the double salt. The first of these animals that died showed no tuberculosis except a small gland and a few tubercles in the spleen. The third also showed but a slight degree of tuberculosis. The second and fourth had a moderately advanced tuberculosis and the last exhibited signs of healed and healing tubercles, as the groins contained small calcified granules and in the lungs and liver the tubercles were hard and fibrous. The controls of this set lived 126, 170, 182, 187, 192 and 205 days, or an average of 177 days. All the controls showed extensive, advanced tuberculosis. If we consider in this experiment the consistently longer life of the treated animals, the less extent of the disease, and the tendency to healing shown in the guinea-pig that lived longest, we may conclude that the double salt of methylene green and mer-

<sup>19</sup> Ztschr. f. Chemotherapie, 1914, 2, p. 106.

<sup>20</sup> Biochem. Ztschr., 1914, 65, p. 460.

curic chloride, as well as that of methylene blue and mercuric chloride, shows some beneficial influence on tuberculosis caused by a strain of tubercle bacilli of relatively low virulence. The strain of high virulence was not tested with this compound.

(d) Diazo amino methylene blue ortho toluidin dimercuric acetate was made for me by my chemical assistant, Doctor Kharasch. The method of making this compound and the two following ones will be reported by him later. The chemical formula for it is given in table 1; it contains 41.3% of mercury, and is quite insoluble. Its inhibitory and germicidal power have not yet been determined. Seven guinea-pigs were inoculated subcutaneously, each with 0.05 mg. of the virulent strain 1305. They were fed daily and received one subcutaneous injection. They lived 8, 32, 100, 106, 137, 140 and 148 days, an average of 96 days, and received 7, 29, 87, 92, 119, 121 and 128 mg. of the compound. All except the 2 that died after 8 and 32 days showed a high degree of advanced generalized tuberculosis. The controls of this set lived 51, 83, 83, 94, 127 and 143 days, or an average of 97 days, and all showed marked and extensive tuberculosis of lymph glands, spleen, liver and lungs. This experiment, therefore, would not indicate that this compound of methylene blue has any beneficial action on this virulent tuberculous infection.

(e) Diazo amino methylene blue brom hydrate ortho toluidene di mercuric acetate. The formula for this is given in table 1, and it contains 39.5% of mercury. It is insoluble. Nine guinea-pigs were inoculated subcutaneously with 0.05 mg. of strain 1305 and then given one injection of the compound suspended in oil and a daily feeding of 1 mg. They lived 8, 23, 32, 76, 81, 95, 129, 133 and 170 days, or an average of 92 days, if we omit the accidental death at 8 days. They had received 10, 21, 64, 69, 81, 131, 125 and 145 mg., or an average of 78 mg. per animal. All except the 3 animals dying after 8, 23, and 32 days, had a pronounced general tuberculosis. Hence this compound, like the same compound without the brom hydrate, has no beneficial influence in this virulent infection.

(f) Diazo amino methylene blue ortho phenol mercuric chloride contains 29.7% of mercury. Ten guinea-pigs were inoculated with 0.05 mg. of strain 1305 and were then fed daily 1 mg. and received one injection of the compound suspended in oil. These lived 11, 16, 18, 48, 72, 126, 144, 154, 171 and 174 days or an average of 127 days, after omitting the first two. They received 14, 19, 21, 35, 55, 101, 117, 125, 126 and 129 mg. of the compound, an average of 74.2 mg. The first 2 died from septic abortion and the third from acute pneumonia. Four of the others showed generalized tuberculosis of moderate degree, while the other 3, which were the last to die, exhibited a slight degree of tuberculosis, a few small tubercles in one of the organs or perhaps only an enlarged gland. The controls of e and f lived 62, 125, 125, 128 and 138 days, or an average of 115 days, and all showed advanced generalized tuberculosis.

Concerning the 3 diazo compounds of methylene blue and mercury just described, but little can be said. A number of the treated animals died early, suggesting a toxic and weakening action of the drug. With the 2 o-toluidin compounds d and e, there was no difference between the treated and untreated animals either in length of life or in extent of the disease. The animals treated with the o-phenol compound f lived slightly longer and had less tuberculous involvement than the controls, although they were inoculated with the virulent strain 1305.

The results of the chemotherapeutic experiments with these 24 mercuric compounds have been described as briefly as possible in the foregoing pages. But, in order to compare these results, a brief summary is given in table 3. It will be seen from this table that, as compared with the untreated controls that had been inoculated with the same dose of the same strain of tubercle bacilli, several showed a greater duration of life and less extent of disease. In nearly all of these, the infection was due to a strain of lower virulence, and when the same drug was tested with a strain of high virulence no effect was noticed.

TABLE 3  
SUMMARY OF RESULTS

Mercury Compounds	Strain of Tubercle Bacilli	Length of Life Compared With Controls	Extent of Disease Compared With Controls
Mercurous chloride .....	O. H.	Greater	Same
Mercury sulpho cyanide.....	O. H.	Slightly less	Less
Mercury potassium cyanide.....	O. H.	Sl. greater	Same
Allyl alcohol mercuric acetate.....	1305	Same	Same
Mercuric succinimide .....	O. H.	Greater	Less
Mercuric salicylate .....	M.	Less	Same
Meruro-iodo-hemol .....	O. H.	Slightly less	Same
Mercuriol (Merck).....	O. H.	Less	Slightly less
Mercury phenolphthalein .....	O. H.	Greater	Same
Mercury tetraiod phenolphthalein.....	O. H.	Same	Same
Fluorescein and mercuric chloride.....	O. H.	Less	Same
Trypan blue mercurous chloride.....	O. H.	Same	Same
Trypan blue mercuric chloride.....	O. H.	Same	Same
4-ortho-oxybenzylidene amino phenyl para mercuric acetate .....	O. H.	Less	Same
1-amino-2(p-naphthalin azo phenyl mercuric acetate) 5-sulphonic acid .....	O. H.	Less	Less
Methylene blue and mercuric chloride.....	O. H.	Much greater	Much less
Iod methylene blue and mercuric chloride.....	1305	Same	Same or less
Methylene green and mercuric chloride.....	O. H.	Same	Same
Diazo-amino methylene blue o-toluidin mercuric acetate .....	O. H.	Greater	Less
Diazo amino methylene blue from hydrate o-toluidin mercuric acetate .....	1305	Same	Same
Diazo amino methylene blue o-phenol mercuric chlorida .....	1305	Greater	Less

Only with one set of animals treated with the double salt of methylene blue and mercuric chloride and with a set treated with diazo amino methylene blue o-phenol mercuric chloride has there been a similar beneficial effect on animals inoculated with a virulent strain, and with none of these drugs has the effect been sufficient so that we could speak of having really cured the disease. As is well known, the guinea-pig is far more susceptible to tuberculosis than is the human being and responds to infection with a more rapidly progressive and fatal disease than does man. Hence a treatment that would cure a tuber-

culous guinea-pig would presumably cure man more easily. A drug that has a decidedly beneficial effect in the guinea-pig would probably have a much greater effect in man. It may well be that the disease caused in guinea-pigs by a less virulent strain like the one described in this paper as the "Old Human" resembles far more nearly the disease in man than does the infection with the virulent strains (Miller's or 1305). However that may be, slight as seem the results reported in this paper, they seem sufficient to justify future work with mercurial compounds in the chemotherapy of experimental tuberculosis. Such work with many new organic compounds of mercury is already under way and will be reported at intervals, as results are obtained that seem worthy of presentation.